

Appendix 2B-2: Status Report on ACME Studies on the Control of Hg Methylation and Bioaccumulation in the Everglades

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Status Report on ACME Studies on the Control of Mercury Methylation and Bioaccumulation in the Everglades

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Introduction and findings to date

The ACME project team, led by the US Geological Survey, and currently supported by the Smithsonian Institution, the University of Alabama, and the Louisiana State University, has been investigating the factors leading to the formation of methylmercury (MeHg) and high levels of mercury (Hg) in biota since 1995. During Phase I, ACME examined the biogeochemical controls on mercury methylation and bioaccumulation across the Everglades, at a series of intensively studies sites in LNWR, the former ENR, WCAs 2A, 2B and 3A, and ENP. Conclusions from that study have are discussed in details in former ECRs and the 2005 SFER. Overall, the study pointed to the importance of the microbial Hg methylation process in the translation of Hg deposition into the methylmercury (MeHg) in the food web; identified sulfur contamination as an important (perhaps dominant) control on Hg methylation in the ecosystem; identified the EAA as an important source of S to the ecosystem; identified soil surface “flocs” and sometime periphyton mats as the major sites of Hg methylation; and showed that benthic food webs are dominant in the marsh ecosystem and are the main source of MeHg into fish.

In the current phase of research, the ACME team is investigating specific linkages between current and forecasted changes to the physical, hydrologic and chemical aspects of the Everglades system, and mercury Hg methylation and bioaccumulation. The field studies focus on the impact of water chemistry on methylation, the role of drying and rewetting of soils (including STA soils) in methylation, the role of STA siting and operations on MeHg production and bioaccumulation. Our research directions have also expanded to begin to examine the effect of sulfate and sulfide on other aspects of the Everglades ecosystem, and to begin development of a diagenetic numerical model for net methylation.

For the past five years, the ACME project has used “mesocosms” - *in situ* enclosures of about ~1m in diameter (Fig. 1) – to assess the impact of water chemistry on Hg methylation and bioaccumulation. In the early 2000’s ACME began to examine the dose-response of methylation to Hg loading, and the effect of Hg “age” on methylation and bioaccumulation. These studies addressed the production of MeHg from newly deposited Hg, compared to Hg already stored in soils. We also began to examine in detail the relationships between sulfate loading and methylation. Mesocosm studies 2000-2002 also identified

dissolved organic matter and a key control on Hg methylation and bioaccumulation. By sampling surface soils in a phosphate-dosing study being done by the SFWMD, the ACME team was able to show that even long-term phosphate additions did not significantly effect the production of MeHg in surface soils/flocs. This work is discussed in the 2003 and 2004 ECRs.

Major findings from mesocosm work to date:

- Hg delivered to shallow, saturated wetlands like the Everglades is rapidly delivered to sites of methylation at or near the soil surface
- A higher fraction of the newly deposited spike Hg is methylated in surface soils than is native Hg, suggesting that Hg newly deposited to the Everglades surface is more bioavailable for methylation than previously deposited pools.
- The change in MeHg production in response to a change in Hg loading depends on the timing and magnitude of transport to sites of methylation. In the Everglades, rapid and substantial transport of Hg through the shallow water column to surface soils means that a relatively high fraction of Hg delivered to the water surface is methylated.
- Both sulfate and dissolved organic matter have major effects on methylation. The balance between sulfate and sulfide determines the effect of sulfur on methylation. In the Everglades, methylation is generally highest at 10-50 μM sulfate in surface waters. Dissolved organic carbon affects methylation by holding Hg in solution. It also affects bioaccumulation by limiting bioavailability of MeHg.
- Biogeochemical parameters that influence methylation rates have a much larger effect on the methylation of newly deposited Hg than on older Hg pools.



Figure 1. Left -an example of an ACME mesocosm study at 3A15. Right – top view of a mesocosm.

Current work plan

Mesocosms studies. The current ACME work plan - for FL Fiscal Years 2003/04 through 2005/06 has multiple separate, but linked components. In the first component, mesocosms studies are being used to address multiple objectives. All current mesocosm work is being conducted at central Everglades site WCA 3A-15, a former “hot-spot” for methylation in the Everglades. Sulfate and DOC concentrations at 3A-15 have declined dramatically since the mid to late 1990’s and have resulted in concomitant decreases in MeHg in soils and fish. Mesocosm work at 3A-15 will help assess the relative impact of SO₄, DOC, and declines in Hg deposition on changes in MeHg production and bioaccumulation.

Objectives of current mesocosm studies:

- Determine quantitatively the individual and synergistic effects of mercury, sulfate and dissolved organic carbon loading on MeHg.
- Determine the methylation efficiency for recently deposited Hg relative to Hg stored in soils.
- Evaluate a new hypothesis - that external sulfate loading (and subsequent accumulation of sulfide in porewater) leads to toxicological impacts on indigenous emergent plants, which may be in part responsible for the proliferation of cattail.
- Evaluate the impact of Fe concentration on Hg methylation and bioaccumulation. This new direction is an expansion of the original scope of work, and evolved from our measurements of Fe biogeochemistry in the ecosystem, and work published on Fe effects in other ecosystems.

Long-term monitoring of core ACME sites. The ACME study has examined Hg and MeHg concentrations in multiple matrices at the core ACME sites since 1995.

Objectives for long-term monitoring:

- Monitor long-term changes in Hg accumulation, net MeHg production and bioaccumulation at the core ACME sites in LNWR, WCA 2A, 2B, 3A and ENP.
- Examine associated biogeochemical parameters to help provide mechanistic explanations for observed changes

Modeling net methylation. The ACME team, in conjunction with Reed Harris, E-MCM developer, is developing a predictive model for MeHg production in the Everglades, including the Stormwater Treatment Areas. The current version of E-MCM uses empirical relationships between ecosystem production, water chemistry and methylation to model methylation. We seek to put methylation into a mechanistic framework, based on microbial activities in soils, and the complexation chemistry of Hg in soils.

To develop a predictive methylmercury model, the following studies are needed:

- Additional detailed down-core measurements of soil biogeochemistry in variety of locations in the Everglades WCAs and STAs.
- Laboratory experiments to further refine our understanding of the fundamental factors that lead to high levels of MeHg formation following drying and rewetting experiments.
- The creation of a numerical diagenetic model that links mercury-sulfur-carbon and can be used as a predictive tool for MeHg formation.

Dry/Rewet Cycles: Examining MeHg production in the Stormwater Treatment Areas. STAs have been and continue to be constructed across the northern Everglades, primarily for the removal of nutrients from runoff waters that in part discharge to the Everglades. The full utilization of these STAs is important aspect of the overall Everglades restoration plan, and achieving its goals. The SFWMD and the State of Florida seek information that would allow management of STAs to reduce or eliminate excess MeHg production events.

Previous research by the ACME team, and observations by SFWMD, show that drying and rewetting cycles can exacerbate the formation of MeHg in the Everglades and in STAs. The 2001 ECR provides information on a strong pulse of MeHg formation in WCA 3A after the 1999 drought. The 2002 ECR provides information from the SFWMD on wetting and redrying in WCA 2A. Experimental work by the ACME team using soils from STA-2 Cell 1 and from WCA 3A-15 show that drying of soils results in release of sulfate, which then “feeds” Hg-methylating sulfate-reducing bacteria upon rewetting (see the 2004 ECR, Appendix 2B-1). However, once sulfide (the end-product of microbial sulfate reduction) begins to accumulation in soils interstitial waters, MeHg production is slowed.

Water levels in the STAs are manipulated for various purposes. Current management practice allows STA cells that are sensitive to MeHg production (for example STA 2 Cell 1) to be held wet in order to minimize the MeHg production that follows drying and wetting cycles. Current research seeks to determine how much drying is tolerable in different soil types, how source waters and soil types impact the drying/rewetting cycle for Hg, and the timing of MeHg pulses following rewetting of the STAs. This work is being accomplished by dry and rewetting soils from different STAs and WCAs under controlled conditions in the laboratory, and be detailed biogeochemical examination of soils in the STAs. This work also provides input data for the numerical modeling effort. These studies will provide information for the management of STAs and the siting of future STAs and reservoirs.

Objectives for current dry/rewet studies in the STAs:

- Examine the timing of sulfate oxidation, sulfide production and Hg methylation in experimental drying and rewetting studies.
- Examine the effects of soil chemistry on the magnitude of MeHg production during dry/rewet cycles. The focus will be on reduced S, organic matter and

Fe in soils. This will be accomplished through a survey of soil geochemistry, Hg and MeHg in STA soils, and with experimental drying and rewetting studies with various STA soil types.

- Use the above information along with the ACME diagenetic model for methylation, to predict MeHg concentrations for STA and WCA soils under changing sulfide and sulfate concentrations and to evaluate the efficacy of strategies for STA operation and siting.

Status and results to date under current work plan

Mesocosms studies.

Hg/SO₄/DOC mesocosms. In the summer of 2003, a large mesocosm study was begun at site 3A15, in which the interactions between sulfate and DOC loading were examined, along with relative methylation rates of newly-deposited Hg was measured relative to previously stored Hg. This study addresses the following objectives:

- Determine quantitatively the individual and synergistic effects of mercury, sulfate and dissolved organic carbon loading on MeHg.
- Determine the methylation efficiency for recently deposited Hg relative to Hg stored in soils.

Twenty mesocosms were emplaced during the summer of 2003. Dosing of the mesocosms began in June. Figure 2 (top) shows the design for this study. Ten mesocosms were used for sulfate plus mercury additions, at five different dosing levels, each in duplicate, and all with a single mercury level of roughly 22 $\mu\text{g}/\text{m}^2$. The target sulfate dosing levels were approximately 4, 8, 12, 16, and 20 mg/l (final concentration in surface water). Four mesocosms (duplicates at each of 2 dosing levels) were used to examine the effects of DOC, again all with a 22 $\mu\text{g}/\text{m}^2$ Hg dose. DOC for the experiment was isolated previously from Everglades surface waters by Dr. George Aiken at USGS Boulder. The original design called for six +DOC mesocosms, but Dr. Aiken was able to isolate enough DOC to dose 4 mesocosms. All +SO₄ mesocosms were dosed approximately monthly (when possible) from June 2003 until June 2005. DOC and Hg additions were one-time events.

All mesocosms, plus two outside control sites were sampled in June, August and November 2003, November 2004, and in June 2005. Additional background samples outside the mesocosms were taken on other dates. Planned sampling in summer 2004 had to be cancelled because of low water levels. The length of the study, and number of sampling points, is more than was called for in the SOW, because conditions in the field prevented airboat access, and hence sulfate dosing, during the long dry period of 2003-2004. The original study design called for an end to the experiment in Nov 2003, but the team agreed to sampling into late 2004, because initial data suggest that sulfate levels in the mesocosms did not stabilize at their new levels until fall of 2003 (after the first sampling points), and the site was not accessible again until summer 2004. We chose to keep dosing the mesocosms with

sulfate, when accessible, during the winter of 2004/2005, so that a summer sample could be collected in 2005. The study ended after a June 2005 sampling.

Everglades Site 3A-15 - Slough

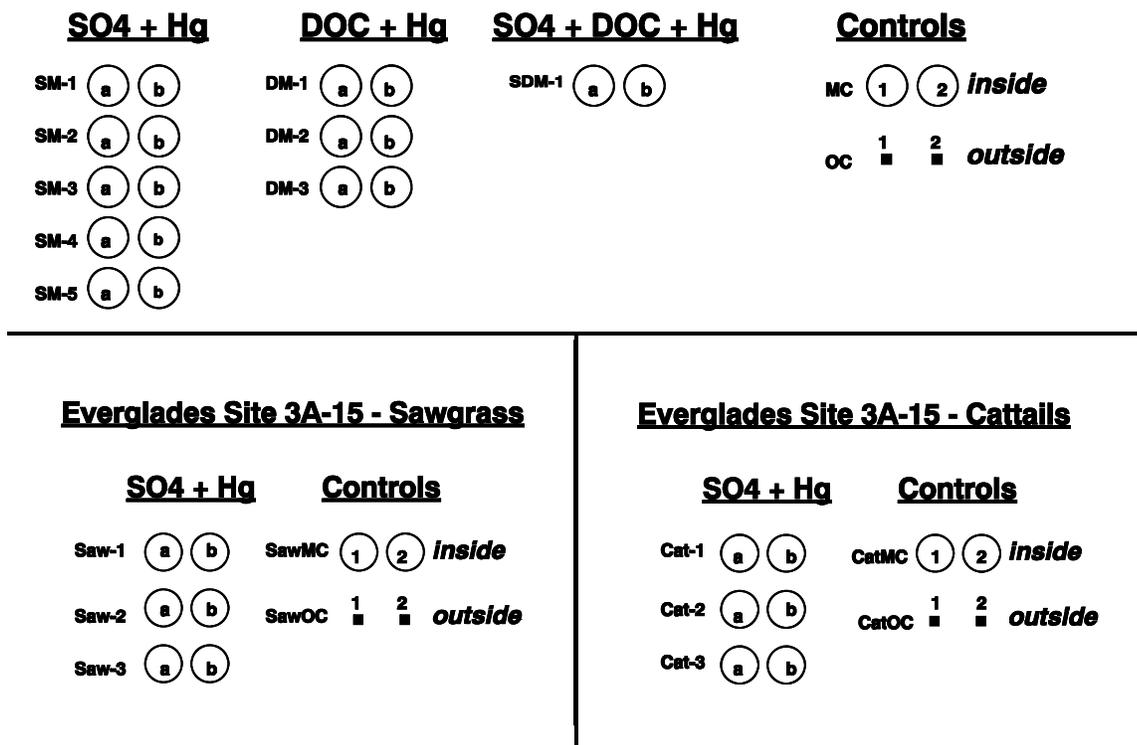


Figure 2. Design for two 3A15 mesocosm experiments, begun in 2003. *Top*: Hg X SO4 X DOC mesocosm study. *Bottom*: Sulfur toxicity study.

Results to date: sulfate levels stabilized at or just below target levels by fall 2003. Fig 3 shows the preliminary average sulfate concentration from monthly sample dates June 2003 through Nov 2004. Note that mesocosms were not accessible during all months. The production of Me202Hg from the 202HgCl2 spike was strongly dependant on mesocosm treatment - both sulfate and DOC concentration. Fig. 4 shows preliminary data on the concentration of MeHg in surface soils/floc on three sampling dates in 2003. Although there is significantly variability between duplicate mesocosms, the optimal sulfate concentration for methylation at 3A15 under current conditions appears to be in the 4-10 mg/L (~40-100 uM) range. This sulfate concentration is well above current concentrations of < 1 mg/L sulfate.

Dissolved organic matter also stimulated methylation production in surface soils. There appear to be interactions between the effects of DOC and sulfate. More data on the

interactions between sulfate and DOC, and the timing of DOC and sulfate effects will be available once samples from later time points are analyzed.

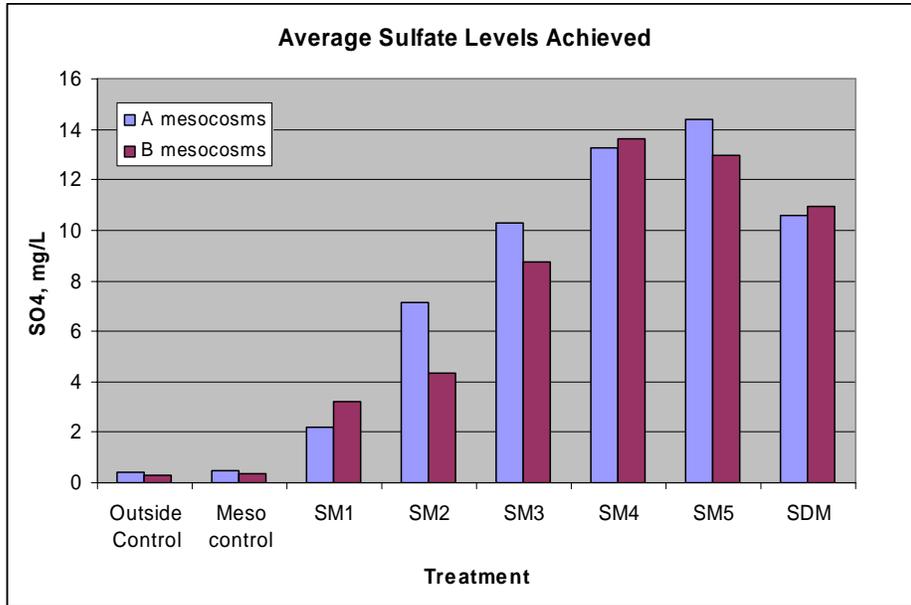


Fig 3. Average surface water sulfate levels achieved in 3A15 mesocosms, June 2003 through June 2005. Data from Orem/USGS.

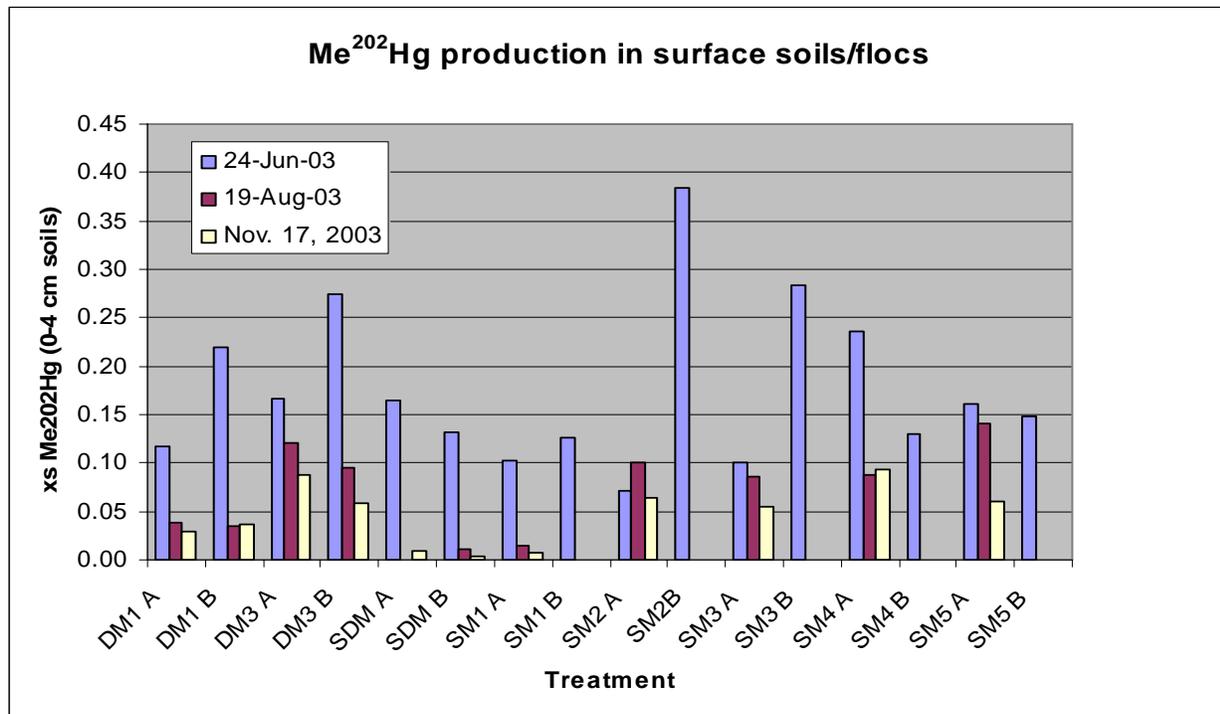


Fig 4. Me²⁰²Hg in surface soils (0-4) on three dates after the initial Hg, sulfate and DOC spikes in June 2003. Data from Gilmour/SERC.

S toxicity mesocosms. Twenty four mesocosms were emplaced at 3A15 in spring and summer 2003 for the plant sulfur toxicity study. The study plan was adjusted from the scope or work to increase the number of replicates for each treatment from two to three, based on statistical needs for analysis of plant effects. Half of the mesocosms are in a cattail stand, the other half are in an adjacent sawgrass stand, but downstream from a tree island (see attached diagram). This mesocosm set is less than a mile from the slough mesocosms. In each type of vegetation, there are triplicate mesocosms at each of three target sulfate levels: 20 mg/l, 50 mg/l, and 100 mg/l; plus three control mesocosms, and three “outside” control sites.

The initial sulfate dosing of these mesocosms occurred in November 2003. All +SO₄ mesocosms have been dosed approximately biweekly since Nov. 2003, except during periods of low water when access was limited. Initial mesocosm sampling occurred in Nov. 2003. Soil sampling focused Hg, MeHg and S concentrations in “outside” controls, in order to minimize disturbance of plants in mesocosms. These mesocosms will be dosed for at least two years, in order to allow plant communities to respond to the sulfate doses. Surface water, pore water and vegetation sampling have taken place 2-3 times per year. Soil sampling will occur approximately iannually during the study.

Iron addition mesocosms. In March, 2005, ten enclosures were moved and re-emplaced at 3A15 for a new Hg addition study, in which the effects of Fe(III) will be examined through Fe(III) amendments. Site background data was collected in March 2005. Fe(III) and Hg amendments were one time only, in June, 2005. Samples were taken just prior to the Fe(III) addition, three days later, and then again during the week of Aug 15, 2005. Sample analysis will be completed during winter 2005-2005.

Long-term monitoring of ACME cores sites

Biogeochemical sample at most of the core ACME sites took place in June 2003 and Nov. 2004 Everglades (Lox, ENR, F1, U3, 2BS, 3A15, 3A33 and 3ATH, plus STA2) and August 2005 (F1, U3, 2BS, 3A15, 3A33 and 3ATH). Sampling in ENP has been limited because a sampling permit is pending.

An example of temporal and spatial trends in DOC in surface waters is given in Fig. 5.

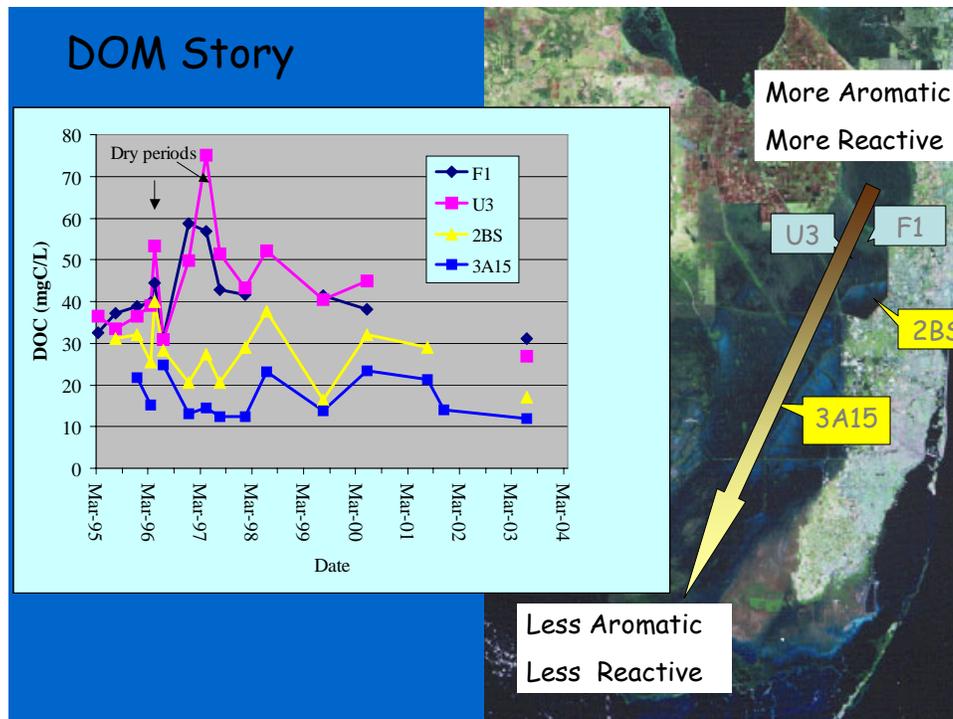


Fig 5. Surface water dissolved organic carbon (DOM or DOC) concentrations at 4 ACME stations from 1995 through 2004. From G. Aiken, USGS

Modeling net MeHg production

To date, work in this area has focused on intensive biogeochemical data collection in support of model development. During the week of May 16th 2004, three cells in STA 3 / 4 were sampled intensively in order to produce the biogeochemical measurements needed for the model. Measurements included microbial rates (sulfate reduction, CO₂ and CH₄ production, and Fe(III) reduction), and detailed solid and pore water chemistry with depth at each site, as described in the SOW. Sampling sites were in open-water areas roughly in the center of each cell. STA 3 / 4 was chosen because little information on soil chemistry is available for this STA, and because it was recently constructed and flooded (winter 2003/4). Dr. Roden and a student worked with us on this sampling effort. Additional WCA sites were sampled in June 2005, and a few more STA sites will be examined in fall 2005. Numerical model development will follow data analysis.

Additionally a meeting was held to compare the Hg addition studies being done in the Everglades with a whole-lake Hg addition study being done in Canada. The ACME/METAALICUS comparison meeting was held in conjunction with the METAALICUS project meeting in Boulder, CO, March 2005. METAALICUS is a whole-watershed Hg addition study in a boreal lake. Comparison of the studies provides insight on similarities and differences in the responses of the ecosystems. Many of the same techniques

are being used in each location; and both locations are being modeled using the diagenetic model under development by Roden et al.

Dry/Rewet studies

A first round of soil drying and rewetting studies was conducted in 2002 using soils from STA2 Cell 1 and WCA 3A15. Results from this experiment confirmed observations from field studies that drying and rewetting of Everglades' soils produce large pulses of MeHg. Significant increases in MeHg in sediments and surface water were observed in dried and rewet cores from both the central Everglades and STA sites, relative to *in situ* concentrations. Sulfate concentrations also increased dramatically in the overlying water following rewetting of the dried cores from both sites. This increase in sulfate was similar to what had been observed in field studies following the 1999 drought and burn in the northern Everglades. This result supports the hypothesis that MeHg production is stimulated in rewet soils by oxidation of organic matter and reduced sulfur pools in sediments during drying periods. This observation agrees with the data collected during the natural drought and rewetting of 1999.

Minimization of drying events in the STAs is a management tool that can be used in STAs that are prone to MeHg production. STAs most prone to MeHg production appear to be those that have not been previously used for agriculture. Very high levels of reduced S in STAs constructed on former agricultural soils, like the former ENR, inhibit MeHg production through the formation of sulfide and Hg-sulfide species that are not available to microorganisms for uptake and methylation. However, iron, cations, organic matter, and other soil chemistry affect the relationships between sulfate reduction, sulfide accumulation, and methylation. Further examination of soils chemistry across the STAs, and the development of a numerical, diagenetic simulation of methylation, are needed to adequately predict the effects of drying and rewetting on MeHg production in these systems.

Detailed biogeochemical measurements of STA soil were begun in 2004 (see above), and are ongoing in 2005. Additional drying and rewetting studies will be conducted with different soil types in the winter of 2005/2006.